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Effects of Vitamin A Supplementary in the Feed to Reduce Toxic Effects of Aflatoxin B_1 on Japanese Quails *(Coturnix coturnix Japonica)*

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Abstract: A study was conducted to determine the efficacy of the antioxidant vitamin A, for reducing aflatoxicosis in Japanese quail (*Coturnix coturnix Japonica*) from hatch to 35 d of age. Sixty Japanese quail chicks were randomly divided into four groups, three treatments and one control. Control group was fed a basal diet while the treatment diets were supplemented with vitamin A (15,000 IU/kg feed), vitamin A (15,000 IU/kg) + low level of aflatoxin B_1 (100 μg/kg) or aflatoxin B_1 (100 μg/kg). The liver was swollen and a bright yellow color; microscopically fatty metamorphosis, necrotic areas and infiltration of polymorphonuclears were observed in aflatoxin B_1 group and was not seen in any other groups. Compared to control, aflatoxin B_1 (AFB_1) reduced body weight gain (BWG) and feed conversion rate (FCR) by 9.3 and 7.6 % respectively. The addition of 15,000 IU/kg of vitamin A without aflatoxin B_1 improved quail growth performance. Dietary vitamin A had affect on BWG and feed consumption ($P<0.05$) and reduced the toxic effects of AFB_1, addition resulted less toxicity in the liver and kidney than AFB_1 group. FC was found significantly important during third and fourth weeks in vitamin A and AFB_1 added group ($P<0.05$) FCR was also better than AFB_1 group. The concentration of liver function enzymes serum glutamic pyruvic transaminase (GPT), serum glutamic-oxaloacetic transaminase (GOT), alkaline phosphatase (ALP) increased when AFB_1 was added to the diet, whereas the supplementation of the diet Vitamin A partially decreased this negative effects. These results demonstrate that vitamin A plays a complex role in the process of chemical aflatoxicosis and when added at 15.000 IU/kg in the diet can provide protection against the harmful effects of AFB_1 for experimental period in Japanese quail.

Key words: Feed, Japanese quail, vitamin A, aflatoxin B_1 Toxicity

Introduction

Mycotoxins are poisonous of several species of molds aflatoxin, one of the most important toxins is a metabolite from the growth of the fungus *Aspergillus flavus* on corn kernels. Problems from mycotoxins have been increasingly observed in all types of animal feeds. All ages are effected but young animals are most susceptible to the effects of aflatoxin. Clinical signs include gastrointestinal dysfunction, reduced productivity, and decreased feed utilization efficiency. Aflatoxin can cause embryonic death, liver damage, decreased reproductive performance, reduced BWG or egg production, and immune system function, in all poultry species, even when its low levels are consumed. Although it generally takes relatively high levels to cause mortality, low levels can be detrimental if continually fed. As a general rule, growing poultry should not receive more than 20 μg/kg aflatoxin in the diet. Chronic exposure to aflatoxins may significantly alter productivity, which can mean the difference between profit and loss to the poultry industry (Hamilton, 1984) AFB_1 can reduce the level of Vit A *in vivo* and this effect on Vit A is correlative with induction of hepatic microsome cytochrome P450 (Liu and Zhou, 1989). On the other hand, vitamin A is a potent biological antioxidant and it significantly prevents aflatoxin induced alterations in the tissue such as liver, kidney and gizzard of chicks. Considerable interactions exist between vitamin A and aflatoxin. In essence vitamin A is anti-mutagenic, both *in vivo* and *in vitro* to prevent aflatoxin induced liver damage. Carotenoids are also effective in reducing DNA damage but less effective than vitamin A. Various studies have demonstrated toxin binding compounds such as vitamins E and C (Hoehler and Marquardt, 1996). However, no data are currently available on the ability of these to prevent aflatoxin toxicity in chicks. Therefore, this study was conducted to evaluate the ability of vitamin A to reduce toxicosis of aflatoxins in Japanese quail.

Materials and Methods

Sixty day-old Japanese quail chicks of both sexes (8 male 7 female for each group) *(Coturnix coturnix japonica)* were randomly divided into four groups of 15 chicks each. Each chick was put into individual stainless steel cages after being weighed. The lighting pattern was 24 hours light and the room was electrically heated. Chicks received the experimental diets and water *ad libitum* for 35 days. Quail were fed a commercial corn-soybean meal based diet (without added antibiotics,

coccidiostatics, or growth promoters), that provided or exceeded nutrient levels recommended by the National Research Council (NRC, 1984), and contained 22 % of crude protein and 3200 ME kcal/kg. AFB_1 was obtained from Sigma company and Vitamin A as all-trans retinol. The basal diet, Table 1, was used in all treatments. There were of four dietary treatments; 1) Control 2) basal diet plus 15,000 IU/kg vitamin A 3) basal diet plus 100 μg/kg aflatoxin and 4) basal diet plus 15,000 IU/kg vitamin A and 100 μg/kg AFB_1. Quail were individually weighed weekly, feed consumption was recorded weekly and mortality was recorded as it occurred. Quail chicks were examined each morning and evening for signs of toxicosis. After 35 days, the study was terminated and all birds were slaughtered. Liver, kidney, gizzard and spleen were collected and checked by routine tissue analysis methods (Anonymous, 1974). The concentrations of GPT, GOT, ALP were determined (Anonymous, 1974). Statistical analysis were conducted using the SPSS 6.0 (1993) program for Duncan. Differences between means were determined using one-way analysis of variance.

Table 1: Composition of basal diets of experiment (%)

Ingredients and analysis	
Corn	56.49
Soybean Meal[1]	25.30
Fish Meal[2]	8.15
Sunflower Oil[3]	4.22
DCP	0.84
Ground limestone	1.15
DL-Methionine	0.60
Salt	0.20
Vitamin mix[4]	0.50
Trace mineral mix	1.0
Lysine	1.20
Sodium	0.15
Chloride	0.20
Calculated analysis	
Crude protein	22.00
ME, kcal/kg	3200

[1]Contains 44 % crude protein [2]Contains 65 % crude protein [3]Contains 8800 ME, kcal/kg [4]Contains vitamin A, 7500 IU; vitamin D, 2500 IU; vitamin E,30 IU; vitamin K_3, 1,65 mg; Thiamine 1.65 mg; Riboflavine 6 mg; pantothenic acid, 9 mg; Niacin, 50 mg; Pyridoxine, 3 mg; Folic acid, 0.75 mg; Choline, 440 mg; vitamin B_{12}, 0.015 mg.

Results and Discussions

Effects of dietary vitamin A and AFB_1 on body weight gain (BWG), feed consumption (FC), and Feed Conversion Rate (FCR) are shown in Table 2.

The most sensitive clinical sign of chronic aflatoxicosis is reduced rate of growth of young animals. Other signs include prolonged clotting time, increases in serum glutamic oxalacetic transaminase Pier (1991). The liver is considered the principal target organ for aflatoxins and in this investigation livers were swollen and a bright yellow color; microscopically fatty metamorphosis, necrotic areas and infiltration of polymorphonuclears were observed in AFB_1 group and have not seen in any other groups. In experiment, the higher mortality was (25%) observed in aflatoxin group in last two weeks. During the study, even low level of AFB_1 increased relative liver weights as compared to control and added vitamin A groups. The supplementation of 15.000 IU/kg Vitamin A produced in a 9.3 % increase in BWG in birds. This result agrees with the findings of Aburto and Britton (1998), who reported that dietary vitamin A increased feed consumption and BWF of Japanese quail. Chicks who received 100 μg/kg aflatoxin without vitamin A consumed significantly less feed during this experiment ($P<0.05$). Similarly, vitamin A reduced the toxic effects of 100 μg/kg AFB_1 and FC in 3rd and 4th week ($P<0.05$). BWG were lower in birds fed AFB_1 but was not found significant ($P<0.05$). Compared to control, AFB_1 reduced BWG and FCR by 9.3 and 7.6 % respectively. Parlat *et al.* (1999) reported that BWG and FCR were decreased significantly by AFB_1 treatment compared with control ($P<0.05$). This result agrees with other research on experimental aflatoxicosis in quails Celik *et al.* (2001). Nyandieka and Wakhisi (1993) reported that vitamins can inhibit liver cancer by inducing hepatic microsomal enzymes that metabolize aflatoxins to noncarcinogenic products. Any carcinoma was not found in experimental birds in this investigation. Increase in liver and kidney weight were the main pathological alterations, reported in other species Celik, *et al.* (1997).There was extensive hyperplasia of the smooth endoplasmic reticulum in birds. Effects of dietary vitamin A and AFB_1 on liver function enzymes are shown in Table 3.

At the end of study, GPT was found significantly different in vitamin A than AFB_1 and control group. Control and vit A + AFB_1 group were significantly different than others ($P<0.05$). The data for the GOT was found significantly different in group vitamin A than others ($P<0.05$). At the end of 7 th. week, ALP was found significantly different in vit A group and between vit A and vit A+ AFB_1 ($P<0.05$). Serum constituents even partly were increased in group AFB_1 in this study are indicators of malfunction of liver enzyme systems. Similar results were found by Abo-Norag *et al.* (1995). The administration of low levels of AFB_1 for only 5 weeks caused some pathological and performance changes but was not enough decreasing the metabolic disfunctions in the liver during the experiment. Vitamin A status of animals can influence AFB_1 genotoxic activity *in vitro* and indicate that this phenomenon also occurs *in vivo*. Thus a similar mechanism may be considered for the protective action of vitamin A both *in vitro* and *in vivo* (Decoudu *et al.*, 1992). A similar results were found by Bhattacharya *et al.* (1989) who suggested that vitamin A can afford

Table 2: Effects of vitamin A on BWG, FC, FCR of quail chicks fed diets with and without and 100 μg/kg AFB_1.

Days	7	14	21	28	35
Body weight gain					
Control	26.75^{a}	77.74^{ab}	141.24^{ab}	183.86^{ab}	206.15^{a}
Vitamin A	28.40^{a}	94.34^{c}	151.46^{b}	198.54^{b}	217.53^{a}
Aflatoxin B_1	25.12^{a}	84.33^{b}	136.62^{a}	172.65^{a}	186.88^{b}
Vitamin A+Aflatoxin B_1	25.33^{a}	75.30^{a}	146.23^{ab}	176.20^{a}	208.76^{a}
Feed consumption					
Control	60.93^{a}	225.44^{a}	463.39^{a}	658.26^{a}	823.97^{a}
Vitamin A	65.33^{a}	270.73^{c}	484.60^{c}	670.53^{b}	825.94^{a}
Aflatoxin B_1	58.53^{a}	262.29^{b}	463.18^{a}	623.72^{bc}	817.14^{a}
Vitamin A+Aflatoxin B_1	57.46^{a}	222.89^{a}	475.08^{b}	627.58^{c}	820.66^{a}
Feed Conversion Rate					
Control	2.28	2.90	3.28	3.58	3.99
Vitamin A	2.30	2.87	3.20	3.38	3.80
Aflatoxin	2.33	3.11	3.39	3.61	4.37
Vitamin A+Aflatoxin	2.27	2.96	3.25	3.56	3.93

^{abc}Means within the same column with different superscripts are significantly different (P<0.05)

Table 3: Effects of vitamin A on serum biochemical values of quail chicks fed diets with and without and 100 μg/kg AFB_1 (IV/I)

Groups	GPT	GOT	ALP
Control	15.49^{c}	184.47^{b}	13.58^{bc}
Vitamin A	13.79^{a}	173.29^{a}	11.71^{a}
Aflatoxin B_1	14.85^{bc}	184.32^{b}	13.81^{c}
Vitamin A+Aflatoxin B_1	14.15^{ab}	182.27^{b}	12.79^{b}

^{abc}Means within the same column with different superscripts are significantly different (P<0.05).

protection against adverse effects of the potent hepatocarcinogen AFB_1. Mettlin (1984) reported that data derived from epidemiologic studies on human populations are consistent with the protection from cancer afforded by vitamin A seen in animal studies. In the present study no mortality have seen in groups given vitamin A. Previous studies have shown that colon epithelium from vitamin-A-deficient rates binds more AFB_1 than colon epithelium from normal, vitamin-A-supplemented animals. In the present study, vitamin A supplementation to the birds given AFB_1, significantly decreased the binding capacity of AFB_1. Gradelet *et al.* (1998) reported that carotenoids exert their protective effect through the deviation of AFB_1 metabolism towards detoxication pathways. Even small amounts of AFB_1 is injurious because of its detrimental effect on the growth of quails. In the study increases were observed in the liver, kidney and gizzard in AFB_1 group, and the relative liver weight was significantly higher than the other treatment groups. Vitamin A treatment significantly ameliorated the aflatoxin-induced changes. Similar findings were reported by Webster *et al.* (1996) who reported that vitamin A thus may control carcinogenesis by manipulating molecular events at the initiation stage. Yu *et al.* (1994) reported the same observation that although protective effects were seen with several antioxidant vitamins, increased DNA adduct formation was observed with beta-carotene and vitamin E. The administration of vitamin A without AFB_1 improved quail growth performance, and reduced the toxic effects of 100 μg/kg AFB_1 for BWG and FCR and addition resulted less toxicity in the liver and kidney than AFB_1 group. As a result, more studies are needed to understand the mechanism of vitamin A's antioxidant activity in mycotoxicosis.

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